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Incorporating concentration dependence in stable isotope mixing models: a response to Phillips and Koch (2002)

Phillips and Koch (2002) suggested that the standard, linear mixing model used to estimate assimilated diets may produce inaccurate results when animals consume foods that vary markedly in their C:N ratios and elemental concentrations. They presented a concentration-dependent model and used published values on captive and free-ranging bears to illustrate theoretical shortcomings of earlier, concentration-independent models. The assimilated diet estimates for Alaskan bears on the Kenai Peninsula using their concentration-dependent model differed markedly from previously published results (Jacoby et al. 1999). For example, the importance of salmon to brown bears (*Ursus arctos*) decreased from 50% to 26% of the assimilated diet and for black bears (*Ursus americanus*) allopatric to brown bears from 53% to zero.

Estimating the importance of salmon to bears has profound conservation implications as salmon stocks become increasingly depleted by human activities. Brown bear populations with significant access to salmon are up to 50 times denser, adult females are twice as heavy, and litter sizes are 25% larger than in salmon-deprived populations (Hilderbrand et al. 1999c). Recently, brown bears have been identified as major conveyors of the marine-derived nutrients in salmon to Alaskan riparian forests (Hilderbrand et al. 1999a; Helfield and Naiman 2001). Thus, the validity of the Phillips and Koch (2002) estimates must be evaluated as they note that incorrect assumptions in mixing models "may produce misleading results with major ramifications on assessments of feeding ecology and management strategies".

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The original dietary estimates of the importance of salmon by Jacoby et al. (1999) and Hilderbrand et al. (1999b, c) used a bear-specific, carbon-enrichment, linear mixing model (Hilderbrand et al. 1996) to separate marine (salmon) from terrestrial carbon sources. This approach is the most direct of all the models, has the fewest assumptions as fractionation effects related to digestion and substrate routing are part of the feeding trial results used to generate the original regression, and has been used previously to estimate the importance of salmon to earlier humans (Chisholm et al. 1982; Taylor et al. 1998). Phillips and Koch (2002) also used a concentration-independent mixing model to simultaneously estimate assimilated dietary salmon, terrestrial meat, and terrestrial plant content and "reached roughly the same conclusions" as Jacoby et al. (1999) and Hilderbrand et al. (1999b, c). Even though the above two estimates are in agreement and are derived from markedly different models and assumptions, Phillips and Koch (2002) insisted that the very different values generated by their concentration-dependent model that requires more inputs and assumptions are the correct values.

The estimates from the Phillips and Koch (2002) concentration-dependent model are driven by the composition and C:N concentrations of the hypothesized assimilated diet, particularly the plant portion of the diet that was composed of equal parts apples (*Malus sylvestris*), blackberries (*Rubus* sp.), domestic cranberries (*Vaccinium macrocarpon*) and raspberries (*Rubus* sp.), and rhubarb stems (*Rheum rhabarbarum*). Despite extensive literature on the diets of bears, Phillips and Koch (2002) clearly made no effort to mimic a realistic plant diet in their modeling efforts as no Alaskan bear consumes their hypothetical diet. Although the actual plant names are irrelevant in illustrating their model, outputs of the model, comparisons to previous estimates, and management implications do depend on the diet chosen. The lack of realistic diet inputs in their example poses serious scientific and conservation concerns.

The plant component of their hypothetical diet has a gross C:N ratio of 44:1 as compared to 4.6:1 and 3.7:1 for salmon and terrestrial meat (Table 1 in Phillips and Koch
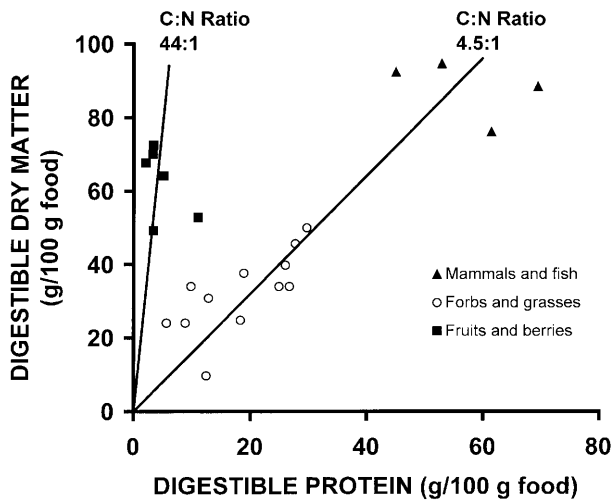
Fig. 1 Digestible dry matter, digestible protein, and assimilated C:N ratios in foods consumed by brown bears and black bears that have been determined either with in vivo feeding trials or by regressions developed between food composition and digestibility. The two *lines* and the assimilated C:N ratios that they represent are the plant average (44:1) used by Phillips and Koch (2002) and the average for grasses, forbs and meats (4.5:1) consumed by bears in several ecosystems. Meat diets are white-tailed deer (*Odocoileus virginianus*), cattle, Columbian ground squirrels (*Spermophilus columbianus*), and cutthroat trout (*Salmo gairdnerii*). Forbs and grasses are white clover (*Trifolium repens*), cow parsnip (*Heracleum lanatum*), peavine (*Lathyrus ochroleucus*), and mixed grasses (*Poa pratensis*, *Phleum pratense*, *and Bromus gracilis*). For plants with multiple values throughout the year, the seasonal extremes were plotted. Fruits and berries include crowberry (*Empetrum nigrum*), elderberry (*Sambucus* sp.), huckleberry (*Vaccinium membranaceum*), lowbush cranberry (*Vaccinium vitis-idaea*), apples (*Malus pumila*) and blueberries (*Vaccinium corymbosum*) (Pritchard and Robbins 1990; McLellan and Hovey 1995; Welch et al. 1997; Rode and Robbins 2000; Rode et al. 2001). Carbon concentrations were estimated as 45% of the plant dry matter (Barbour et al. 1987). Carbon concentrations in animal tissue were estimated as in Phillips and Koch (2002). We assume that the carbon concentration of the digested matter is the same as that in the original food. Digestible protein is the "true" or assimilated nitrogen and is estimated as 92% for plant protein, 100% for meat, and 100% minus the nondigestible protein component of claws and hair for whole mammals (Pritchard and Robbins 1990; Robbins 1993)

2002). This very large difference in the C:N ratios of the plant portion of the diet as compared to the animal portion leads to the very skewed, curvilinear mixing triangle in Fig. 2B of Phillips and Koch (2002). The above plant C:N ratio is characteristic of assimilated fruit and berry diets as most are high in available carbohydrates but very low in nitrogen (Fig. 1; Welch et al. 1997; Rode and Robbins 2000). However, the assimilated C:N ratio of grasses and forbs consumed by bears is indistinguishable from that of animal tissues, including salmon (Fig. 1; Phillips and Koch 2002). This occurs because foliage is much higher in protein (i.e., nitrogen), but the dry matter (i.e., carbon) is poorly digested because bears are unable to digest plant fiber (Pritichard and Robbins 1990).

Thus, to evaluate the dietary estimates proposed by Phillips and Koch (2002), we need to determine if the plant-based diet of any, wild, Alaskan brown or black

bear is entirely fruit and berries. In addition, because the bear tissue used for isotope analyses was hair that begins growing as early as 1 May and ceases growing just prior to hibernation, the bears must consume fruit and berries as the only dietary plant component during the entire year. That is ecologically unrealistic as fruits and berries are not available in amounts needed by bears during the spring and early summer. Even in ecosystems with abundant seasonal meat resources (e.g., salmon), forbs, grasses, and sedges can comprise up to 98% of the diet during other seasons (McCarthy 1989; Rode et al. 2001). Because there is frequently little else to eat, the weight of foliage consumed per day can be enormous (e.g., 41% of body weight in small bears) (Rode et al. 2001). Fruits and berries become available only in mid-summer and fall and generally overlap with salmon availability on the Kenai Peninsula.

Fruits and berries can be an important food resource, particularly for bears without access to salmon, but the small size of many wild berries and their dispersed distribution within the plant create a difficult foraging problem for larger bears (e.g., salmon-feeding brown bears) (Welch et al. 1997; Hilderbrand et al. 1999c). For example, a 360 kg, salmon-feeding brown bear on the Kenai Peninsula would strive to meet its energy requirements by consuming over 260,000 huckleberries (*V. membranaceum*) per day. However, because the three-dimensional distribution of berries in the shrub's architecture will decrease harvest rate, the above brown bear could consume approximately 70,000 berries/day and would lose as much as 1.9 kg/day on a berry-only diet. A single 5 kg salmon has the energy content of over 34,000 huckleberries that would require over 6 h for a bear to harvest. Thus, even though the energy content of the individual berry can be highly digestible and both brown and black bears consume them, berries are not a nutritionally significant food resource for all bears, particularly if salmon are available. Indeed, black bears with unimpeded access to abundant salmon readily use the resource even when berries are available (Reimchen 1998, 2000; Chi 1999).

We agree with Phillips and Koch (2002) that differences in elemental concentration and digestibility between the plant and meat components of an omnivore's diet will reduce the meat proportion and increase the plant component when one extrapolates assimilated diets estimated from concentration-independent models to concentration-weighted, assimilated biomass or actual food habits. However, their assertion that plant matter is undervalued when concentration-independent models are used to estimate the assimilated diet of interior bears also must be evaluated. The previously estimated assimilated dietary content of plant matter using concentration-independent models is 98±2% (1 SD) for interior, adult, female brown bears without access to salmon or abundant large ungulate herds (i.e., Glacier National Park, Cabinet-Yaak mountains of Idaho and Montana, Denali National Park, Columbia River basin of British Columbia, and Kluane National Park of Canada) (Hilderbrand et al. 1999b, c; Jacoby et al. 1999; Hobson et al. 2000). Use of

a concentration-dependent model cannot increase the estimated plant contribution to the diets of these bears as current estimates include 100%. While males in the above ecosystems are more carnivorous (80±12% dietary plant matter), carnivory provides the nutrients necessary for males to be approximately 80% larger than females even though adult weights of both sexes are well below their genetic potential (Hilderbrand et al. 1999c; Rode et al. 2001). The assimilated dietary content of plant matter to black bears of both sexes and all age classes in similar interior areas is 90±4% (Jacoby et al. 1999; Hobson et al. 2000; Partridge et al. 2001). Thus, Phillips and Koch's (2002) suggestion that concentration-dependent models should be used because the dietary importance of plant matter to interior bears is undervalued by concentration-independent models is baseless.

We feel that use of a concentration-dependent model should be a multi-step process as the increasing number of assumptions and inputs required for such models as compared to concentration-independent models will increase the likelihood of an error and decrease the overall accuracy of their dietary estimates. Phillips and Koch's (2002) assumption that berries and fruits are the only plant matter consumed by bears is an example of such an error. These assumptions and errors become especially problematic when studying highly omnivorous bears or when using fossilized remains as the correct application of a concentration-dependent model requires digestibility estimates for each diet component, which Phillips and Koch (2002) neither recognized nor used. Phillips and Koch (2002) incorrectly used the C:N ratios of the foods rather than of the assimilated diet and, thereby, incorrectly suggested in their conclusions that one only needs to "measure and report elemental concentrations for each source" and "use a concentration-weighted mixing model – if the concentrations differ substantially among the sources." That view dramatically understates the challenges in developing and using concentration-dependent models.

For example, use of a concentration-dependent model requires an a priori estimate of the relative proportion of the individual foods in the plant and animal components of the diet (i.e., sources) in order to produce weighted estimates of the assimilated C:N ratios and isotope signatures. This problem has been simplified when using concentration-independent models by using the estimated, weighted isotope signature for plant matter determined from resident herbivore signatures (e.g., Hilderbrand et al. 1999c; Partridge et al. 2001) or the unweighted isotope signature specific for bear foods (Hobson et al. 2000). However, those approaches, even with the caveat that as many individual foods as possible should be analyzed (Jacoby et al. 1999; Hobson et al. 2000), cannot be used to estimate assimilated C:N ratios.

The plant component of a bear's diet can be composed of as many as 2–10 species of berries, 5–30 or more species of herbaceous matter, and several species of tubers and bulbs (Mattson et al. 1991; McLellan and Hovey 1995). Even if one reduces the question to the ratio of the relative intake of fruits and berries, tubers and bulbs, to herbaceous matter, there is no method currently available to determine that ratio reliably in field studies, particularly for those studies using hair or bone to estimate the annual diet. Similarly, the terrestrial and marine animal components, which can include everything from insect larvae to bison and clams to salmon or beached whales, may be simpler to estimate in that the assimilated C:N ratio of animal matter varies much less than that in plant matter, although selective feeding on fat deposits (e.g., salmon eggs or blubber) in comparison to the entire animal will render this estimate much more difficult.

Unfortunately, the above problems pale in comparison to an additional input necessary to use concentration-dependent models as proposed by Phillips and Koch (2002). An estimate of the annual, dietary C:N ratio within each source also requires a measure of the absolute intake (kg) of the foods because intake varies markedly between seasons. For example, the annual assimilated C:N ratio in the plant source for interior bears that consume grasses and forbs (4.5:1) during the spring and summer and fruits and berries (44:1) in the fall would be 24:1 if they were consumed in equal assimilated amounts, but that ratio will range from 11:1 when 5 times more grasses and forbs are assimilated in the spring and summer than fruits and berries in the fall to 37:1 when 5 times more fruits and berries are assimilated in the fall than grasses and forbs in the spring and summer. Seasonal plant matter intake has never been measured in free-ranging bears. Thus, the opportunity to use a concentration-dependent model to understand bear foraging ecology may be very rare and useful only for very simple systems. In truth, we would not need to use stable isotopes to estimate diets if we could accurately provide all of the inputs necessary to use concentration-dependent models.

Finally, the concentration-dependent mixing triangle as used by Phillips and Koch (2002) ignores internal metabolic processes and, thereby, assumes that (1) there are no interaction effects between diet components (i.e., isotope signatures are additive) and (2) internal metabolism homogenizes all carbon and nitrogen so the original dietary ratios are reflected in all tissues, or at least tissues being sampled. One or both of these assumptions may be incorrect for the diet used in their bear example. For example, bears utilize two carbon sinks (respired carbon dioxide and fat accumulation) to metabolically deal with the excess carbon relative to nitrogen when fruit and berry diets are consumed. Energy metabolism for maintenance, and thereby carbon dioxide loss, increases up to 200% when bears consume fruit and berry diets relative to diets with lower C:N ratios (Rode and Robbins 2000; Felicetti and Robbins, unpublished data). Similarly, the composition of the gain can vary from 100% fat when consuming fruit and berry diets to 100% lean mass when consuming diets with more balanced C:N ratios. Thus, the metabolically effective C:N ratio is that of the daily assimilated diet and secondarily that of the individual sources or the yearly diet. Dietary mixing of high carbon/low nitrogen fruits and berries and high nitrogen meat or vegetation is far more complex than depicted by Phillips and Koch (2002).

In summary, we view the development and application of stable isotopes to nutritional ecology studies as a field in its infancy. Several of the weaknesses that we identified in the Phillips and Koch (2002) model also apply to concentration-independent models. Thus, we encourage the development of new approaches and interpretations and support the call of Ben-David and Schell (2001) for more studies of "the relations between diet composition, metabolic pathways, consumer tissue composition, and associated isotopic data." Unfortunately, the bear examples presented by Phillips and Koch (2002) best illustrate that model development and use in a complete vacuum of nutritional, physiological, and ecological knowledge is destined to produce erroneous, misleading results.

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